I. Remarks

Status of Claims

Claims 25-48 are currently pending.

Request for amendment of Specification

The Examiner requested Applicants to amend the title of the specification to recite the claimed antibody of the instant invention. *See* Paper No. 4202006, page 2. Applicants respectfully direct the Examiner to Applicants' response dated February 3, 2006 wherein Applicants amended the title of the invention to "Antibodies To HHPEN62 Polypeptide." If this title is still objected to by the Examiner, Applicants urge the Examiner to provide further guidance.

II. *Rejections of claims 25-47 under 35 U.S.C. § 101*

Claims 25-47 have been rejected under 35 U.S.C. § 101 as allegedly not supported by "either a specific and substantial asserted utility, or a well-established utility." *See*, Paper No. 4202006, page 3. Applicants respectfully disagree and traverse this rejection.

First, Applicants respectfully note that the test for specificity under 35 U.S.C. § 101 is whether an asserted utility is specific to the subject matter claimed, in contrast to a utility that would be applicable to the broad class of the invention, such as use of a complex machine for landfill. *See*, M.P.E.P. §2107 I(A). Accordingly, the disclosure that the instant invention is useful, *inter alia*, in treating, preventing, detecting and/or diagnosing neural and neurodegenerative disorders is specific, as not every antibody may be used to treat, prevent, detect and/or diagnose a neural and/or neurodegenerative disorder. *See*, page 85, paragraph 0197. Consequently, the skilled artisan would most certainly not consider such a use to be a "throw-away utility" such as landfill, and accordingly consider the asserted utilities of the present invention specific.

Second, Applicants respectfully note that where an applicant discloses a biological activity (*e.g.*, "Elevated expression of [HHPEN62] within the frontal cortex of the brain indicates that it may be involved in neuronal survival; synapse formation; conductance; neuronal differentiation, etc," *See*, page 86, paragraph 0197), and reasonably correlates that activity to a disease or condition (*e.g., inter alia*, neuronal and neurodegenerative disorders), the applicant has sufficiently identified a specific utility for the invention. M.P.E.P. § 2107 I(A) at 2100-32

(emphasis added). Stated in other words, so long as the correlation between the biological activity and the asserted use in a particular disease or condition is sufficient to convince one of skill in the art, then the specificity requirement of 35 U.S.C. § 101 is satisfied. *See*, Fujikawa v. Wattanasin, 39 U.S.P.Q.2d 1895 (Fed. Cir. 1996). Applicants submit that, based on the instant disclosure, the skilled artisan would be convinced that the asserted utilities of the instant invention are specific.

For example, the specification discloses that the instant invention is expressed primarily in whole brain tissue, as well as brain specific tissues such as hypothalymus, frontal cortex, cerebellum, amygdala, and hippocampus tissues. *See*, page 84, paragraph 0195. In addition, the specification discloses that the instant invention maps to chromosome 18q22-23, a chromosomal region identified in the art as a susceptibility loci for neuronal and neurodegenerative disorders including bipolar affective disorder. *See*, page 84, paragraph 0194, and Nothen et al. "Evaluation of Linkage of Bipolar Affective Disorder to Chromsome 18 in a Sample of 57 German Families." *Molecular Psychiatry*, 4:76-84 (1999) **(Exhibit A)**. Thus, one of skill in the art would be convinced that the asserted utilities are specific since the instant invention is described as useful to treat, prevent, detect and/or diagnose a neural and/or neurodegenerative disorder, wherein the instant invention is both expressed primarily in the brain and is located in a chromosomal region implicated in neuronal and neurodegenerative disorders.

Third, the disclosed specific utilities discussed above are also substantial, since Applicants have "asserted that the claimed invention is useful for [a] particular practical purpose," wherein Applicants disclose a therapeutic method of treating a known disease, such as neuronal and neurodegenerative diseases. *See both*, M.P.E.P. §2107 I(B)(1); and §2107.01 I(B). Moreover, Applicants respectfully note that pharmacological or therapeutic inventions that provide any "immediate benefit to the public" satisfy 35 U.S.C. § 101. *See*, Nelson v. Bowler, 626 F.2d 853, 856, 206 U.S.P.Q. 881, 883 (C.C.P.A. 1980); *See also,* M.P.E.P. §2107.01(III). It is well-established that the mere identification of a pharmacological activity of a compound that is relevant to an asserted pharmacological use provides an "immediate benefit to the public" and satisfies the utility requirement. *Id.* Accordingly, Applicants respectfully contend that the utilities discussed above and disclosed in the instant specification are clearly substantial.

Finally, the Examiner alleges that the specification does not disclose the role of the protein of the instant invention in any of the conditions asserted by Applicants that may be amenable to treatment using the claimed antibodies, and that the specification does not disclose *in vitro* or *in vivo* experimental results of administering the protein or antibodies. *See*, Paper No.

4202006 pages 5, last sentence, through page 6, first sentence. Applicants respectfully disagree and traverse this rejection.

As a preliminary matter, Applicants respectfully remind the Examiner that Applicants do not have to prove that a correlation exists between a particular activity and an asserted therapeutic use of a compound as a matter of statistical certainty or provide actual evidence of success in treating humans where such a utility is asserted. All that is required of Applicants is that there be a *reasonable* correlation between the biological activity and the asserted utility. *See Nelson v. Bowler*, 626 F.2d 853, 857 (C.C.P.A. 1980).

Applicants also respectfully note that credibility of a specific and substantial utility is assessed from the perspective of one of ordinary skill in the art in view of the disclosure and any other evidence of record. *See*, M.P.E.P. §2107. In addition, the M.P.E.P. further states that "an applicant need only provide one credible assertion of specific and substantial utility for each claimed invention to satisfy 35 U.S.C. 101 and 35 U.S.C. 112, additional statements of utility, even if not 'credible' do not render the claimed invention lacking in utility." *See*, M.P.E.P. § 2107.02(I). As described in detail above, Applicants submit that the specification does assert a substantial and specific utility. In addition, Applicants respectfully submit that the specific and substantial utility asserted by Applicants is credible, given the state of the art and the disclosure of the present application.

As discussed previously, given the detailed description of the structure and activity of the specified polypeptides and the disclosed brain-specific expression pattern and chromosomal localization, together with the well recognized role of chromosome 18 in neuronal disorders, one skilled in the art would have found the asserted utility for the protein of the subject invention credible upon reading the specification

In addition, "to overcome the presumption of truth that an assertion of utility by the Applicant enjoys ... [it must be established] that one of ordinary skill in the art would doubt the truth of the statement of utility...To do this, [the Examiner] must provide evidence sufficient to show that the statement of asserted utility would be considered false by a person of ordinary skill in the art." *See* M.P.E.P. § 2107.02III(A) at 2139-40. Moreover, "an assertion [of utility] is credible unless (A) the logic underlying the assertion is seriously flawed, or (B) the facts upon which the assertion [of utility] is based are inconsistent with the logic underlying the assertion." *See* M.P.E.P. § 2107.02(B) at 2100-40. However, in the instant rejection, the Examiner has not provided such reasoning or evidence. In the present case, the pending Office Action has not made the required showing that any of the disclosed, specific and substantial utilities for the

claimed polypeptides cited above would not be unbelieveable in light of the teachings of the specification.

In view of the above arguments, Applicants have provided evidence and reasoning which supports the Applicants' assertion of utility. In particular, Applicants have provided evidence that the polypeptides and/or antibodies raised against the polypeptide of the instant application are useful, *inter alia*, in treating, preventing, detecting and/or diagnosing neural and neurodegenerative disorders. Accordingly, Applicants respectfully submit that the rejection of claims 25-47, under 35 U.S.C. § 101 has been obviated. Thus, Applicants respectfully request that the rejection of claims 25-47 be reconsidered and withdrawn.

III. ***Rejections of claims 25-47 under 35 U.S.C. § 112***

The Examiner rejected claims 25-47 under 35 U.S.C. § 112, first paragraph because the claimed invention is allegedly "not supported by either a specific and substantial or a well established utility." Paper No. 4202006, page 6. Applicants respectfully disagree and traverse.

Applicants respectfully submit that the Examiner "should not impose a 35 U.S.C. § 112, first paragraph, rejection grounded on a 'lack of utility' basis unless a 35 U.S.C. §101 rejection is proper." M.P.E.P. § 2107.01(IV) at 2100-36. As discussed above, the claimed invention complies with the utility requirement of 35 U.S.C. § 101. Accordingly, Applicants respectfully request that the rejection of claims 25-47 under 35 U.S.C. § 112, first paragraph, be reconsidered and withdrawn.

IV. Conclusion

The Applicant respectfully requests that the aforementioned amendments and remarks be entered and made of record in the file history of the instant application. In view of the foregoing remarks, the Applicant believes that the Examiner's concerns have been fully addressed and that this application is in condition for allowance. An early notice to that effect is urged. The Examiner is invited to call the undersigned at the phone number provided below if any further action by the Applicant would expedite the examination of this application.

Finally, if there are any fees due in connection with the filing of this paper, please charge the fees to our Deposit Account No. 08-3425. If a fee is required for an extension of time under 37 C.F.R. § 1.136 not accounted for above, such an extension is requested and the fee should also be charged to our Deposit Account.

Date: 7/12/06

Respectfully submitted,

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Exhibit A

Molecular Psychiatry (1999) 4, 76–84

ORIGINAL RESEARCH ARTICLE

Evaluation of linkage of bipolar affective disorder to chromosome 18 in a sample of 57 German families

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Keywords: manic depression; gene localization; genetics; sib pair

Previously reported linkage of bipolar affective disorder to DNA markers on chromosome 18 was reexamined in a large sample of German bipolar families. Twenty-three short tandem repeat markers were investigated in 57 families containing 103 individuals with bipolar I disorder (BPI), 26 with bipolar II disorder (BPII), nine with schizoaffective disorder of the bipolar type (SA/BP), and 38 individuals with recurrent unipolar depression (UPR). Evidence for linkage was tested with parametric and non-parametric methods under two definitions of the affected phenotype. Analysis of all 57 families revealed no robust evidence for linkage. Following previous reports we performed separate analyses after subdividing the families with respect to the sex of the transmitting parent. Fourteen families were classified as paternal and 12 families as maternal. In 31 families the parental lineage of transmission of the disease could not be determined ('either' families). Evidence for linkage was obtained for chromosomal region 18p11.2 in the paternal families and for 18q22–23 in the 'either' families. The findings on 18p11.2 and 18q22–23 support prior evidence for susceptibility loci in these regions. The parent-of-origin effect on 18p11.2 is confirmed in our sample. The delineation of characteristics of 'either' families requires further study.

Although the etiology of bipolar affective disorder is unknown, strong support for an important genetic component comes from the results of family, twin, and adoption studies.[1] Linkage studies of bipolar disorder to date have provided suggestive evidence in favor of locus heterogeneity. Promising chromosomal regions suggested by recent linkage studies include regions on chromosome 18.

Berrettini *et al*[2] first reported linkage of bipolar disorder to a region near the centromere on chromosome 18p in 22 families using the affected-sib-pair (ASP) method and the affected-pedigree-member (APM) method. Parametric LOD score analysis of all 22 families revealed negative LOD scores. However, individual families yielded LOD scores >1 assuming dominant or recessive genetic models. Confirmatory evidence for a bipolar susceptibility locus in this chromosomal region was found by Stine *et al*.[3] Both parametric LOD score analysis and ASP analysis supported linkage in their study of 28 families. In addition, the same study reported a second susceptibility locus on the long arm of chromosome 18 (18q21). Interestingly, linkage to loci on both 18p and 18q was strongest in those families, in which the father or one of the father's siblings was affected, suggesting a parent-of-origin effect operating in bipolar disorder. Gershon *et al*[4] re-analyzed the 18p marker data of Berrettini *et al*[2] by the sex of the transmitting parent. Although no kindred with limited paternal transmission was observed, ASP analysis yielded highly significant excess allele sharing in the pedigrees with mixed maternal-paternal transmission (in different pedigree branches) but not in pedigrees with exclusively maternal transmission confirming the observation of Stine *et al*.[3] Further evidence for a bipolar susceptibility locus on the long arm of chromosome 18 was provided by four recent linkage studies. Freimer *et al*[5] analyzed two large Costa Rican families. Linkage disequilibrium based methods suggested a locus in 18q22–23, whereas the LOD score analysis provided only weak support for the presence of linkage. De bruyn *et al*[6] found linkage of bipolar disorder to markers in 18q21.33–q23 in a large Belgian family using both LOD score and ASP analyses. Coon *et al*[7] obtained weak evidence for linkage at 18q23 in a sample of 13 families from Utah. LOD score analyses revealed positive LOD scores for three markers in this region. No positive results were obtained with APM and ASP analyses. Using LOD score analysis, Ewald *et al*[8] reported linkage of markers to a more proximal part on the long arm of chromosome 18 (q12) in two Danish families. In a study of a 30-family sample, McMahon *et al*[9] obtained confirmatory evidence for a locus at 18q21 using ASP analysis. A parent-of-origin effect was observed, but it was not consistently paternal. No strong evidence for a potential locus on chromosome 18 was found in the recent collaborative NIMH study on bipolar affective disorder.[10] Only one marker at chromosome 18p11.2 and one marker at 18q23 yielded a slight elevation in allele sharing. It is clear from simulations of additive oligogenic inheritance that expectations of universal agreement regarding reported linkages to bipolar disorder are unrealistic.[11] Suarez *et al*[11]

have shown that a much smaller family sample may be sufficient to initially detect linkage to one of the trait loci than to replicate linkage to that locus. Therefore, it might not be unexpected that some other studies have not been able to support linkage with chromosome 18.[12–16] On the other hand, linkage results must be replicated to be credible. If results from two or more independent studies provide significant evidence for linkage in independent series of pedigrees, it is reasonable to assume validity.[17] In the current study we wanted to test the validity of the recent linkage reports investigating a large series of 57 small to moderate German pedigrees. We analyzed 23 microsatellite markers from chromosome 18. The majority of markers were localized on 18p11.2 and 18q21.2–q23; the regions on chromosome 18 being most consistently implicated by previous studies.

LOD score analysis

Twenty-three markers were genotyped on chromosome 18. The LOD scores are shown in Table 1. Overall two-point LOD scores did not exceed 1.0 for either model, except for marker D18S37 (1.08 at $\theta = 0.1$) under a recessive genetic model and ASM II. When we divided the pedigrees into maternal, paternal, and 'either' families, LOD scores >1 were seen in the paternal families for markers D18S453 (1.91 at $\theta = 0.0$), D18S37 (1.67 at $\theta = 0.0$), and D18S40 (1.65 at $\theta = 0.01$) under a recessive genetic model and ASM I, and for D18S53 (1.06 at $\theta = 0.0$) under a dominant genetic model and ASM I. Under a recessive model and ASM II, marker D18S453 yielded a LOD score of 1.34 at $\theta = 0.0$. In the maternal families LOD scores were below 1.0 for all models. In the 'either' families LOD scores >1 were obtained for D18S469 (1.17 at $\theta = 0.0$) and D18S70 (1.12 at $\theta = 0.05$) under a dominant model and ASM II, for D18S554 (1.59 at $\theta = 0.05$) and D18S70 (1.65 at $\theta = 0.05$) under a dominant model and ASM I, and for D18S554 (1.26 at $\theta = 0.1$) and D18S70 (1.09 at $\theta = 0.1$) with a recessive model and ASM I.

Parametric multipoint LOD score analyses showed peaks in the same regions where two-point LOD scores >1 had been observed (Figure 1): a LOD score of 2.54 was observed in the paternal families at markers D18S37, D18S453, and D18S40 using a recessive genetic model and a stringent phenotype definition. The non-parametric Z-all score in this region peaked at 1.34 ($P = 0.03$) (Figure 1). For the 'either' families, we obtained a maximum parametric LOD score of 2.1 at markers D18S554 and D18S461 under a dominant model and ASM I. The Z-all score was 2.20 ($P = 0.0006$) (Figure 1).

Affected-sib-pair analysis

ASP analyses in all families showed excess allele sharing for D18S40 ($P = 0.035$) under ASM II and a non-significant trend for flanking markers. In the paternal and maternal families, we observed excess allele sharing for the following markers: D18S453 ($P = 0.054$) under ASM II in the paternal pedigrees and D18S36 ($P = 0.0064$) under ASM II in the maternal pedigrees. In the 'either' families, we found excess allele sharing for D18S71 under ASM II. Using ASM I excess allele sharing was observed for D18S554 ($P = 0.017$) and D18S70 ($P = 0.032$).

Many reports of linkage in psychiatric disorders have been published in recent years. Interpretation of these findings is difficult and requires critical consideration of the study design, eg power of the family sample, informativity of markers, and statistical methods. The application of statistical thresholds[17] as guidelines for the interpretation of linkage findings alone may not be sufficient to reflect the complex situation in multifactorial disease.[18,19] Therefore, until susceptibility genes are identified, replication of linkage findings will be the most important criterion to assess their validity.

Although in our study LOD scores failed to reach 3, our results do seem to provide some support for the existence of susceptibility loci on both the short and long arm of chromosome 18. Interestingly, positive findings were obtained after subdividing the families according to the gender of the disease-transmitting parent. Our linkage data support the modest evidence for linkage with markers on 18p11.2 originally reported by Berrettini *et al*.[2,20] In accordance with the results of Stine *et al*[3] and Gershon *et al*[4] we found the highest LOD scores in the subset of paternal families. Moreover, our multipoint LOD score peaked at the same loci (D18S37, D18S453, D18S40; Figure 1) where Gershon *et al*[4] and Stine *et al*[3] found their highest LOD scores on 18p11.2. Thus, this is the third study with a large family sample to find evidence for a susceptibility locus for bipolar disorder in a relatively narrow region on the short arm of chromosome 18. Interestingly, in the collaborative NIMH study the marker D18S40 was the only marker on 18p which showed significantly elevated allele sharing.[10] Unfortunately, no data have yet been published from this sample where the sample was subdivided according to the sex of the transmitting parent.

A paternal effect operating in bipolar disorder is also supported by clinical data.[21–23] Grigoroiu-Serbanescu *et al*[21] found that paternal transmission of the disease was associated with a significantly younger age of onset of bipolar illness in probands. McMahon *et al*[23] observed an increased rate of major affective disorder among maternal relatives and a higher than expected proportion of families with no paternal transmission.

A possible biological explanation for the paternal effect observed in the linkage studies is that a susceptibility gene on 18p underlies genomic imprinting. An alternative explanation might be differences between male and female recombination rates on chromosome 18.[3,24,25] Given a simple fully penetrant dominant or recessive mode of inheritance, it is clear that different allele sharing should occur in paternal and maternal families, simply if the male and female recombination rates between disease and marker locus are different. If, for instance, the male recombination rate is higher than the female recombination rate, one would expect higher sharing in the maternal families for a dominant

Table 1 Results of parametric LOD score analyses

Marker	*Genetic map*		*Dominant broad*				*Dominant narrow*			
	Female	*Male*	*All pedigrees (n = 57) Lod (theta max.)*	*Paternal (n = 14) Lod (theta max.)*	*Maternal (n = 12) Lod (theta max.)*	*Either (n = 31) Lod (theta max.)*	*All pedigrees (n = 57) Lod (theta max.)*	*Paternal (n = 14) Lod (theta max.)*	*Maternal (n = 12) Lod (theta max.)*	*Either (n = 31) Lod (theta max.)*
PACAP	0.000	0.000	–	0.02 (0.4)	–	–	–	–	–	–
D18 S 62	0.104	0.5	–	–	0.06 (0.4)	–	–	–	–	0.03 (0.3)
D18 S 53	0.5	0.135	0.32 (0.2)	–	–	0.23 (0.2)	0.05 (0.3)	**1.06 (0.0)**	–	–
GOLF	0.01	0.001	0.35 (0.2)	–	0.66 (0.1)	0.43 (0.1)	0.08 (0.3)	0.09 (0.2)	0.14 (0.2)	–
D18 S 71	0.014	0.02	0.17 (0.3)	0.21 (0.01)	0.10 (0.2)	0.49 (0.05)	0.07 (0.3)	0.36 (0.1)	0.01 (0.3)	0.06 (0.3)
D18 S 37	0.001	0.021	0.62 (0.1)	0.16 (0.1)	0.10 (0.2)	0.60 (0.05)	0.02 (0.3)	0.57 (0.1)	0.18 (0.0)	–
D18 S 453	0.001	0.001	0.49 (0.2)	0.30 (0.1)	0.03 (0.3)	0.37 (0.2)	–	0.47 (0.1)	–	–
D18 S 40	0.025	0.008	0.44 (0.2)	–	0.33 (0.05)	0.48 (0.1)	–	0.39 (0.2)	–	–
D18 S 44	0.030	0.015	0.15 (0.3)	–	0.43 (0.2)	0.01 (0.4)	–	0.07 (0.2)	–	–
D18 S 36	0.141	0.028	0.49 (0.2)	–	0.78 (0.1)	0.15 (0.2)	–	0.01 (0.3)	0.30 (0.1)	–
D18 S 39	0.702	0.211	0.04 (0.3)	–	–	0.37 (0.2)	–	–	–	0.09 (0.3)
D18 S 41	0.029	0.008	0.26 (0.2)	0.01 (0.3)	0.03 (0.3)	0.42 (0.1)	–	–	–	0.10 (0.3)
D18 S 64	0.054	0.023	–	–	–	0.17 (0.2)	–	–	–	–
D18 S 38	0.011	0.015	–	–	–	0.12 (0.2)	–	–	–	0.27 (0.2)
D18 S 1147	0.012	0.034	–	–	–	0.65 (0.1)	0.16 (0.3)	–	–	**1.06 (0.1)**
D18 S 68	0.014	0.058	–	–	–	0.25 (0.2)	–	–	–	0.54 (0.1)
D18 S 392	0.001	0.001	–	–	–	0.45 (0.2)	–	–	–	0.32 (0.2)
D18 S 541	0.125	0.87	0.01 (0.4)	–	–	0.78 (0.1)	–	–	–	0.12 (0.3)
MBP	0.132	0.090	–	–	–	0.55 (0.1)	–	–	–	0.90 (0.05)
D18 S 469	0.119	0.048	0.02 (0.3)	0.01 (0.3)	–	**1.17 (0.0)**	–	–	–	0.75 (0.1)
18 S 554	0.161	0.064	–	–	–	0.81 (0.1)	0.18 (0.3)	0.06 (0.3)	–	**1.59 (0.05)**
D18 S 461	0.010	0.082	0.43 (0.2)	0.06 (0.2)	–	0.67 (0.1)	0.25 (0.2)	–	–	0.85 (0.05)
D18 S 70	0.014	0.109	0.31 (0.2)	0.11 (0.2)	–	**1.12 (0.05)**	0.78 (0.2)	0.11 (0.3)	–	**1.65 (0.05)**

Continued

Table 1 Continued

Marker	Genetic map		Recessive broad				Recessive narrow			
	Female	Male	All pedigrees (n = 57) Lod (theta max.)	Paternal (n = 14) Lod (theta max.)	Maternal (n = 12) Lod (theta max.)	Either (n = 31) Lod (theta max.)	All pedigrees (n = 57) Lod (theta max.)	Paternal (n = 14) Lod (theta max.)	Maternal (n = 12) Lod (theta max.)	Either (n = 31) Lod (theta max.)
PCAP	0.000	0.000	–	–	0.14 (0.2)	–	0.01 (0.4)	–	0.38 (0.1)	–
D18 S 62	0.104	0.5	–	–	–	0.05 (0.3)	–	–	–	–
D18 S 53	0.5	0.135	0.17 (0.2)	0.30 (0.05)	–	0.05 (0.3)	–	0.96 (0.05)	0.01 (0.4)	–
GOLF	0.01	0.001	0.48 (0.1)	0.05 (0.2)	0.03 (0.2)	0.14 (0.2)	0.28 (0.2)	0.78 (0.05)	0.08 (0.2)	–
D18 S 71	0.014	0.02	0.76 (0.1)	0.83 (0.0)	–	0.99 (0.0)	0.18 (0.3)	0.80 (0.05)	0.06 (0.3)	0.01 (0.4)
D18 S 37	0.001	0.021	**1.08 (0.1)**	0.25 (0.1)	0.11 (0.1)	0.50 (0.1)	0.32 (0.2)	**1.67 (0.0)**	0.32 (0.05)	–
D18 S 453	0.001	0.001	0.75 (0.1)	**1.34 (0.0)**	0.01 (0.4)	0.27 (0.2)	0.30 (0.3)	**1.91 (0.0)**	0.16 (0.2)	–
D18 S 40	0.025	0.008	–	0.22 (0)	0.56 (0.01)	0.14 (0.2)	0.40 (0.2)	**1.65 (0.01)**	0.39 (0.05)	–
D18 S 44	0.030	0.015	0.16 (0.3)	–	0.01 (0.4)	–	–	0.89 (0.05)	–	–
D18 S 36	0.141	0.028	0.10 (0.3)	–	0.32 (0.1)	0.17 (0.2)	–	0.09 (0.2)	0.07 (0.2)	–
D18 S 39	0.702	0.211	0.50 (0.2)	–	0.02 (0.3)	0.13 (0.2)	0.24 (0.3)	0.15 (0.2)	–	0.19 (0.2)
D18 S 41	0.029	0.008	–	–	0.21 (0.1)	0.25 (0.2)	0.33 (0.2)	0.02 (0.3)	0.44 (0.01)	0.12 (0.3)
D18 S 64	0.054	0.023	0.01 (0.4)	–	–	0.12 (0.2)	–	0.02 (0.3)	–	–
D18 S 38	0.011	0.015	0.12 (0.3)	–	–	0.13 (0.2)	–	–	–	0.22 (0.2)
D18 S 1147	0.012	0.034	0.12 (0.3)	–	–	0.28 (0.2)	0.27 (0.3)	0.02 (0.3)	–	0.68 (0.2)
D18 S 68	0.014	0.058	0.01 (0.4)	–	–	0.36 (0.2)	0.07 (0.3)	–	–	0.62 (0.2)
D18 S 392	0.001	0.001	–	–	–	0.43 (0.2)	0.01 (0.4)	–	–	0.38 (0.2)
D18 S 541	0.125	0.87	–	–	–	0.68 (0.1)	–	–	–	0.10 (0.3)
MBP	0.132	0.090	–	–	–	0.46 (0.2)	0.19 (0.3)	–	–	0.87 (0.1)
D18 S 469	0.119	0.048	0.13 (0.3)	–	–	0.82 (0.05)	0.25 (0.2)	–	–	0.98 (0.1)
D18 S 554	0.161	0.064	0.46 (0.2)	–	0.20 (0.1)	0.84 (0.1)	0.47 (0.2)	–	–	**1.26 (0.1)**
D18 S 461	0.010	0.082	0.45 (0.2)	–	0.30 (0.1)	0.42 (0.1)	0.05 (0.3)	–	–	0.60 (0.2)
D18 S 70	0.014	0.109	0.31 (0.2)	–	0.07 (0.2)	0.91 (0.1)	0.82 (0.2)	0.15 (0.2)	–	**1.09 (0.1)**

Note: LOD scores are given for two disease models (broad and narrow) and two genetic models (dominant and recessive). Results are shown for all pedigrees and separate analyses of 'paternal', 'maternal', and 'either' families. Only positive LOD scores are reported (theta max. < 0.5). The genetic map was calculated by means of the LINKMAP program of the LINKAGE package.[36]

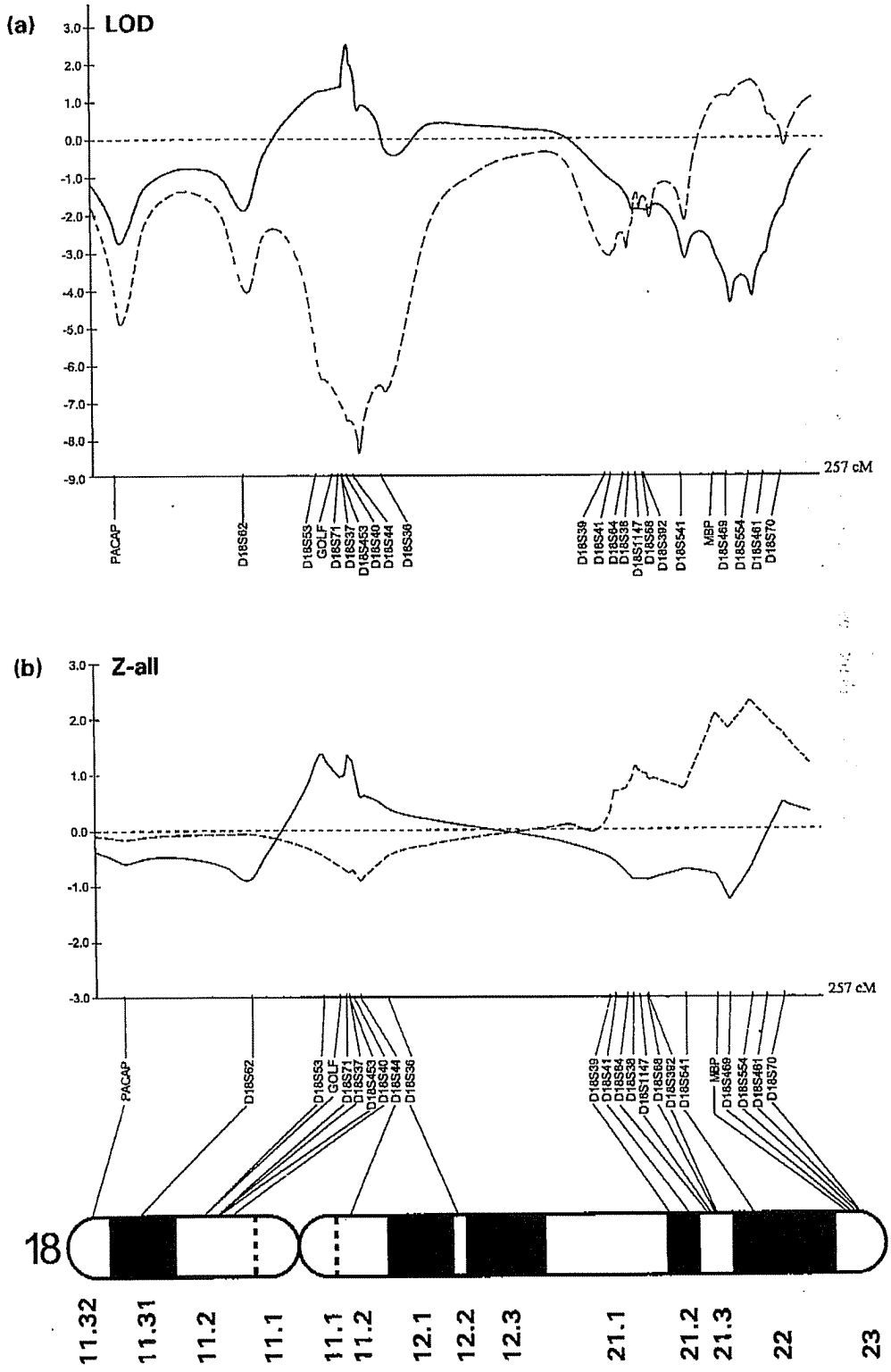

Figure 1 (a) Parametric and (b) non-parametric multipoint linkage analyses of chromosome 18 markers in paternal (straight line) and 'either' (dashed line) families.

model and higher sharing in the paternal families for a recessive model. Under a dominant model, information about linkage between disease and marker locus mainly comes from the transmission of marker alleles from the affected parent to the affected sibs. That means that the information about linkage is in the transmission from father to affected sib in paternal families and in the transmission from mother to the affected children in the maternal families. If we now assume the male recombination rate to be higher, more evidence for linkage is expected to come from the maternal families since here the recombination rate for the relevant transmissions is lower. If a recessive mode of inheritance is assumed, information is mainly contributed by the non-affected parent (ie, from the paternal side in the maternal families and vice versa). Consequently, in this situation a higher male recombination rate will reduce the sharing rate in the maternal families.

To investigate the potential effect of different recombination rates we performed a simulation study. Based on the genetic models used in our analyses we generated 1000 simulations of 100 extended sib pair families. In addition to the marker and disease information of the affected sibs and their parents we assumed to have disease information on the grandparents. This imitates the real situation where in the case when both parents are unaffected the disease status of grandparents is used to assign a family to the paternal or maternal group, respectively. In our simulations we assumed a male recombination rate of 5% and a female recombination rate of 20%. Table 2 shows the allele sharing observed in the different types of families (paternal, maternal, and either families). As expected, allele sharing was higher in paternal families for the dominant models and higher in maternal families for the recessive models. However, when we looked at the power to detect a significant deviation from random allele sharing we found that the power was always higher in the combined data set than in the different subsamples (Table 2). In addition to the evidence given by the simulation study there are other arguments that the positive findings in paternal pedigrees are not simply due to sex differences in recombination. If the findings were caused by this mechanism, one would expect a plethora of falsely positive linkage reports in this region of 18p and not expect an inflation in the number of negative linkage reports, because such linkage studies would presumably detect the same increased non-specific allele sharing. But several such negative reports have appeared.[12–16] Also, when the male and female recombination rates are compared across different regions of chromosome 18, the largest discrepancies occur on 18q.[24,25] Discrepancies on 18p near the centromere are much smaller. Our linkage analyses of markers from the long arm of chromosome 18 provide some support for a second susceptibility locus for bipolar disorder on chromosome 18. The chromosomal region 18q22–23 has originally been proposed by Freimer *et al*[5] who studied two large pedigrees from a genetically homogeneous population in Costa Rica. Their systematic genome screen revealed three markers on chromosome 18 (D18S64, D18S61, and D18S1161) that exceeded a LOD score threshold of 1.6. Fine mapping in this region with additional markers using association analyses produced significant *P*-values for D18S469, D18S554, D18S461, and D18S70. Except for D18S461, these markers yield LOD scores >1.0 in the 'either' subgroup of our family sample as well. Allele sharing IBD was >50% for all four markers with significant *P*-values for D18S554 and D18S70.

Surprisingly, evidence for linkage to 18q22–23 is restricted to the 'either' subgroup of our families. A comparison of results between paternal and 'either' families reveals opposite findings for these two subgroups. While the paternal families are linked to 18p11.2 and display exclusion of linkage to 18q22–23, we observe exclusion of linkage to 18p11.2 in the 'either' families and evidence for linkage to 18q22–23. A possible interpretation of these results is that two different genetic transmission patterns are separated to a considerable extent by the division of pedigrees into maternal, paternal, and 'either' families. Initially, we simply considered the 'either' families a sample which could not be classified into maternal or paternal families. However, if the 'either' families were just a mixture of paternal and maternal families that cannot be separated because of limited pedigree size we would actually not expect the observed positive linkage finding on 18q22–23. Neither paternal nor maternal families give evidence for linkage in this area which makes it unlikely that a mixture of both produces a positive result. Comparison of the family structures between the

Table 2 Simulation of effects of sex differences in recombination rates

Genetic model	*Allele sharing*				*Power*			
	Paternal families	*Maternal families*	*Either families*	*All families*	*Paternal families*	*Maternal families*	*Either families*	*All families*
Dominant broad	64% ± 7%	57% ± 7%	58% ± 6%	59% ± 5%	71%	30%	40%	83%
Dominant narrow	69% ± 5%	59% ± 6%	64% ± 7%	64% ± 3%	96%	45%	66%	99%
Recessive broad	60% ± 9%	63% ± 8%	61% ± 5%	61% ± 4%	39%	53%	77%	93%
Recessive narrow	63% ± 10%	70% ± 9%	75% ± 4%	72% ± 3%	50%	75%	100%	100%

subgroups shows that the parent's affection status is a prominent difference: in contrast to maternal or paternal families where in most cases one parent is affected, the main proportion of the 'either' subgroup (27 out of 31 families) represents families where none of the probands' parents has a psychiatric diagnosis that is included in our disease models. This prominent family structure in the subgroup of 'either' families may reflect a recessive gene action. Surprisingly, we found the most significant results using a dominant inheritance model. Further studies of independent family samples may help to clarify this issue. Of course, there may be other, not yet recognized characteristics that distinguish between 'either' and maternal/paternal families.

In accordance with Freimer *et al*,[5] De bruyn *et al*,[6] and Coon *et al*[7] our results support a bipolar locus at 18q22–23. Other linkage results on the long arm of chromosome 18 have been reported by Stine *et al*[3] and McMahon *et al*[9] on q21 and Ewald *et al*[8] on q12. Similar to our study, Stine *et al*[3] reported evidence for two bipolar susceptibility loci on chromosome 18. The exact location of the putative disease locus on 18q may be difficult to determine due to genetic heterogeneity and the probably low relative risk conferred by this locus. Therefore, the possibility cannot be ruled out that all linkage studies on 18q detect the same disease locus. Indeed, subsequent ASP analyses of the Costa Rican kindreds[26] revealed nominally significant allele sharing at D18S64, one of the most significant markers in the report by Stine *et al*.[3]

We note that our linkage findings on chromosome 18 should be interpreted in the light of statistical testing on the two genetic models and the two affected status models. Likewise, the same data are scrutinized using several analytical methods. Clearly, multiple testing has an inflationary impact on *P* levels. However, we believe that there is no clear way of correcting for these effects, which are common to many linkage analyses of complex traits.

Any single study will be insufficient to provide convincing proof for a susceptibility locus in a complex disease because of unknown mode of inheritance, genetic heterogeneity, and nongenetic factors. Although our results do not allow definitive conclusions, they are consistent with the hypothesis that susceptibility loci are present on the short and long arm of chromosome 18. It might well be that for a complex disease such as manic depression it would be unrealistic to expect to obtain much stronger evidence from a data set of this size and that definitive conclusions will need to be based on consideration of results from many independent sources.

Material and methods

Family ascertainment

Families were recruited in six clinical centers (Wurzburg, Mainz, Haar, Dresden, Munchen, Bonn) in Germany. Standard diagnostic definitions for ascertainment and extension are the following: bipolar I (BPI), schizoaffective, bipolar type (SA/BP), unipolar, recurrent (UPR), unipolar, single episode (UPS) and other minor psychiatric disorders are defined by DSMIII-R criteria;[27] bipolar II (BPII) by Research Diagnostic Criteria (RDC)[28] with the modification that it requires recurrent episodes of depression. The diagnosis of BPII cannot be made in DSMIII-R, but is made in RDC. We additionally specified that depression be recurrent because of concerns about the reliability of hypomania and single-episode major depression.[29]

Inclusion criteria for the systematically ascertained BP families were: (1) a proband with BPI and admission to one of the treatment facilities screened; (2) a secondary affected sib with either BPI, BPII, SA/BP, or UPR; and (3) availability of both parents or—if only one parent was accessible—availability of at least two more sibs from the sibship of the proband.

All individuals were interviewed by an experienced psychiatrist using the Schedule for Affective Disorders and Schizophrenia—Lifetime Version (SADS-L).[30] Best estimate diagnoses were based on the interview, review of all available clinical records and family history information.

Description of families

The family sample consisted of 385 individuals from 57 families. None of these families had previously been included in a linkage study with markers from chromosome 18. Results from a subsample of the present sample have previously been published for markers on chromosome 12.[31] The mean number of individuals per pedigree was 6.8. The distribution of diagnoses was as follows: 103 individuals with BPI, 26 individuals with BPII, nine individuals with SA/BP, 38 individuals with UPR, and 38 individuals with a minor psychiatric diagnosis. One hundred and seventy-one individuals were unaffected.

Except for five families (WUE49, WUE67, WUE78, MAI18, HAA117), there were no families where both parents of the index case had a psychiatric diagnosis. In family WUE49, the father had a diagnosis of BPI, and the mother had a diagnosis of UPR. The father of family WUE78 displayed an adjustment disorder with depressed mood, the mother had UPS. The father of family WUE67 had UPR, the mother was BPI. In family MAI18 the father was diagnosed with UPS, the mother with BPII. Both father and mother of family HAA117 had a personality disorder. There were 14 'paternal families', in which the father of the proband, the grandfather or grandmother on the paternal side or at least one of the father's sibs was affected with BPI, BPII, SA/BP, or UPR, respectively. Similarly, we classified 12 families as 'maternal'. For the remaining 31 families, the parental lineage of transmission of the disease could not be determined. These families were designated 'either' families. Twenty-seven of these families could not be classified, because none of the parents was affected and there were no parental relatives available or there were no affected relatives in either parental lineage. In two of the families with two or more branches (MA121, DRE19), we observed both maternal

and paternal transmission of the disease and therefore classified them 'either'. The four families with both parents of the index case having a psychiatric diagnosis were classified as follows: families WUE49, WUE78, and HAA117 were treated as 'either' families, MAI18 was a maternal family. The family classifications were made blind to linkage results.

The 14 paternal families included 102 individuals: 29 BPI, nine BPII, one SA/BP, 12 UPR, four with a minor psychiatric diagnosis and 47 unaffected. In the 12 maternal families, there were 98 individuals: 24 BPI, 10 BPII, one SA/BP, 14 UPR, nine with a minor psychiatric diagnosis and 40 unaffected. The 31 'either' families consisted of 185 individuals: 50 BPI, seven BPII, seven SA/BP, 12 UPR, 25 with a minor psychiatric diagnosis and 84 unaffected.

DNA isolation and cell lines

EDTA anticoagulated venous blood samples were collected from 320 individuals who were available for the study. Leukocyte DNA was isolated as described.[32] Whenever possible, leukocytes were isolated and transformed using Epstein–Barr virus in order to establish permanent cell lines.

Genotyping

Complete genotypic data were collected from 320 individuals. We studied 23 short tandem repeat (STR) markers on chromosome 18 (PACAP, D18S62, D18S53, GOLF, D18S71, D18S37, D18S453, D18S40, D18S44, D18S36, D18S39, D18S41, D18S64, D18S38, D18S1147, D18S68, D18S392, D18S541, MBP, D18S469, D18S554, D18S461, D18S70). Markers D18S64, D18S68, D18S469, and D18S70 were taken from the ABI Prism Linkage Mapping Set (Applied Biosystems, Foster City, CA, USA). Information on primer sequences for all other markers was taken from published marker maps[33–35] (Genome Data Base Version 5.6).

One oligonucleotide of each pair of PCR primers was fluorescein labeled. The PCR reaction for each marker was carried out in a 10-μl volume containing 40 ng genomic DNA, 5 pmol of each primer, 200 μM of each dNTP, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5–2.5 mM $MgCl_2$, and 0.6 U Taq polymerase (Life Technologies, Rockville, MD, USA). After an initial denaturation of 5 min at 95°C, 33 cycles of amplification of 15 s at 94°C, 15 s at 55–62°C, and 30 s at 72°C were performed in a Perkin Elmer 9600 thermocycler. The resulting amplified products were separated on 4% denaturing polyacrylamide gels on an automated DNA sequencer (Model 377, Applied Biosystems). Allele sizes were determined relative to an internal size standard in each lane using Genescan Analysis and Genotyper software (Applied Biosystems). In addition, a reference individual with known genotype was loaded on each gel. All gels were scored independently by two individuals who were blind to the disease status. Each marker in every family was tested for Mendelian inheritance.

Linkage analyses

Two models of affection were used in the linkage analyses: affection status model (ASM)-I (narrow definition of the phenotype) included only individuals with BPI as affected, all other psychiatric diagnoses were coded as 'unknown'; ASM II (broad definition) included all individuals with a diagnosis of BPI, BPII, SA/BP, and UPR with UPS and other minor psychiatric disorders coded 'unknown'.

There are about 115 ASPs in our 57 pedigrees. This number of ASPs has a power greater than 90% to detect linkage ($\theta \leq 0.05$) at a level $\alpha = 5\%$ under ASM II (recessive and dominant) if at least 50% of the kindreds are linked to a single locus.

Two-point LOD scores were calculated by means of MLINK and ILINK programs of the LINKAGE package.[36] All analyses were conducted using an 'affecteds-only' approach. For both disease definitions the LOD scores were calculated assuming both a dominant and a recessive mode of inheritance. Under the broad affection model we assumed a phenocopy rate of 3.2% and a penetrance of 50% under both genetic models. The frequency of the disease allele was set to 24.5% and 3% for the recessive and dominant genetic model, respectively. For the narrow affection model the phenocopy rate was set to 0.1%, penetrance was assumed to be 50% and the frequency of the disease allele was set to 13.4% under recessive and to 0.9% under dominant mode of inheritance. These assumptions correspond to a lifetime morbid risk of 6% for diseases included in ASM II and of 1% for BPI (ASM I).[37] An age-dependency of the penetrance was not taken into account, since using 'affecteds-only' treats unaffected individuals as phenotype unknown, which diminishes the effect of quantifying the correct penetrance.

The *P*-values for allele sharing between affected sibs were derived by comparing the observed sharing rate for a marker with the exact distribution of allele sharing expected under the null hypothesis of no linkage given the number and size of sib-ships which were informative of the marker. The (one-sided) *P*-value was taken as the probability to have the observed or a higher sharing rate under the null hypothesis.

Multipoint LOD scores as well as nonparametric multipoint analyses were performed using the GENEHUNTER program Version 1.1.[38] For the multipoint LOD score analysis, the same models as for the two-point analysis were used (dominant and recessive mode of inheritance, narrow and broad disease model). For non-parametric multipoint linkage analysis both pairwise identical by descent (IBD), allele sharing (Z-pairs) and IBD sharing among all affected family members (Z-all) was calculated. Only results from the Z-all statistics are given because testing whether the same allele is found IBD in many affected relatives is a more powerful strategy than considering one relative pair at a time.[38]

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